processes. Long lasting production of ROS results in the accumulation of DNA damage both in nucleous and mitochondria. Although caloric restriction has been shown to retard aging processes and increase lifespan of various organisms, the underlying mechanism remains unknown. To clarify the relationship between mitochondrial energy transduction and ROS generation, properties of liver mitochondria from young and aged male Wistar rats were analyzed with and without giving starvation. Kinetic analysis using a high sensitive chemiluminescence probe L012 revealed that ROS generation associated with succinate oxidation of mitochondria differed significantly with their respiratory states; ROS production by mitochondria was higher with young (19-week-old) than with aged (two-year-old) rats. Mitochondrial generation of ROS decreased significantly after starvation of both animal groups. Similar results were also obtained with gulutamate oxidation. Recent studies demonstrate that cellular generation of ROS increased prior to the onset of mitochondrial permeability transition and DNA fragmentation. Thus, we analyzed the ROS generation by Ca^{2+}-loaded mitochondria and its relationship with their swelling and cytochrome c release. We found that the ROS generation by control rat liver mitochondria was greater than that by caloric restriction group. These results suggest that caloric restriction affects mitochondrial generation of ROS and sequence of events leading to apoptosis in the liver of young and aged subjects. Effects of dietary ubiquinone and related compounds on mitochondrial ROS generation and cytochrome c release will be discussed.

P13-164

LIPID, GENOMIC AND MITOCHONDRIAL DNA OXIDATIVE DAMAGE CAUSED BY THE ALTERATIONS OF HYPOTHALAMIC-PITUITARY-GONADAL AXIS IN THE BRAIN OF MALE AND FEMALE RATS

B. Marzani,[1] *S. Giardina,*[1] *M. Tonini,*[1] *and F. Marzatico*[1]

[1]***Department of Physiological-Pharmacological Sciences, Section of Pharmacology and Pharmacological Biotechnologies, University of Pavia, Piazza Botta 11-27100 Pavia, Italy***

During aging neuroendocrine functions undergo changes, particularly to the hypothalamic-pituitary-gonadal (HPG) axis. The aim of this study was to analyse whether HPG axis alterations were associated with changes of brain oxidative status. Basal and stimulated lipid peroxide (LPO) levels, genomic and mitochondrial 8-hydroxy-2′-deoxyguanosine (8-OHdG) content, were detected in the hippocampus and the cortex of sham-operated controls and gonadectomized Wistar rats of both sexes. In the hippocampus, castration increased basal LPO levels, while in stimulated LPO levels were unaffected. Both these parameters remained unchanged in the hippocampus of ovariectomized female rats. In the cortex, castration did not modify basal and stimulated LPO levels. In the cortex of ovariectomized female rats basal LPO content was enhanced, whereas no change was found after stimulation. In the hippocampus, castration reduced while ovariectomy increased 8-OHdG content in genomic DNA; no changes were observed in mitochondrial DNA. In the cortex, gonadectomy increased 8-OHdG content in both genomic and mitochondrial DNA. To conclude, HPG axis alterations were associated with lipid and DNA oxidative changes in the rat hippocampus and cortex. A different response of the two brain areas and the two sexes to modifications of gonadal hormone levels was observed.

P13-165

MODERATE EXERCISE RETARDS NEUROLOGICAL IMPAIRMENT, OXIDATIVE STRESS AND DECREASED MITOCHONDRIAL ELECTRON TRANSFER ASSOCIATED TO AGE

A. Navarro,[1] *N. Escudero,*[1] *J. Fernández-Carrión,*[1] *C. Piñuela,*[1] *and A. Boveris*[2]

[1]***Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Cadiz, Spain,*** [2]***School of Pharmacy and Biochemistry, University of Buenos Aires, Argentina***

Moderate exercise training in a treadmill, from 28 wk to 78 wk of age, extended male and female mice survival with medium lifespan increases of 19 % and 9 % respectively, accompanied by increased performance in behavioural tests (tightrope and T-maze tests) at 52 wk of age, in males and females. In brain, heart, liver and kidney at 52 wk of age, moderate exercise significantly decreased the aging-associated development of oxidative stress by preventing: 1) the increase in protein carbonyls and TBARS contents of submitochondrial membranes; 2) the decrease in antioxidant enzyme activities (mitochondrial and cytosolic superoxide dismutase, Mn-SOD and Cu, Zn-SOD, and catalase) and 3) the decrease in mitochondrial NADH-cytochrome c reductase and cytochrome oxidase activities. These effects were no longer significant at 78 wk of age. Moderate exercise increased lifespan and increased neuromuscular function and exploratory performance, likely by decreasing oxidative stress and by protecting mitochondrial functions from the age-associated decrease in mitochondrial enzyme activities.

P13-166

HISTOLOGICAL MITOCHONDRIAL MARKERS OF TO AGING IN MICE HIPPOCAMPUS

A. Navarro[1] *and C. Piñuela*[1]

[1]***Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Cadiz, Spain***

Mitochondrial markers were determined by histochemical and inmunohistochemical techniques in the hippocampus of aging rats with the aim of establishing an association between the markers and both normal aging and age-related neurodegenerative disorders. Nitric oxide synthase activity (mtNOS and nNOS) was determined by staining for NADPH-diaphorase (NADPH-d), and mitochondrial

electron transfer activity by staining for NADH dehydrogenase (NADH-d), and by immunohistochemical analysis of cytochrome c with a polyclonal antibody. Both NOS (mtNOS and nNOS) and NADH dehydrogenase activities significantly decreased upon aging, as well as cytochrome c content. In the hippocampus of young animals NADPH labelling was found in the pyramidal cells of CA and the hilus. Furthermore, intensive stain of the neuropil was observed in CA and the polimorphic layer. NADH-dehydrogenase was located in the cells of CA (CA1, CA2, CA3), hilus and the neuropil of the molecular layer. Cytochrome c was localized in the subgranular zone and the outer part of the CA. The staining due to these three mitochondrial markers decreased in aged animals. In most of the cases cells were not stained and the neuropil showed a weak stain for both NOS, NADH dehydrogenase and cytochrome c. The results indicate decreased mitochondrial activities in the aging rat hippocampus, which agrees with the aging-associated impairment of memory and cognitive functions.

P13-167

MITOCHONDRIAL NITRIC OXIDE SYNTHASE AND MITOCHONDRIAL ELECTRON TRANSFER ACTIVITIES AS MARKERS OF AGING

A. Navarro,[1] *H. González,*[1] *J. C. García-Ortiz,*[1] *O. Rojas,*[1] *and A. Boveris*[2]

[1]***Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Cadiz, Spain,*** [2]***School of Pharmacy and Biochemistry, University of Buenos Aires, Argentina***

The decrease of neurological performance in normal aging is directly related to the brain oxidative stress and inversely related to lifespan. Male mice lifespan was increased by 8–10% (median and maximal lifespan, respectively) in mice with high spontaneous neurological activity, by 21–15% after moderate exercise; and by 25–20% after supplementation with vitamin E. Oxidative stress markers, TBARS and protein carbonyl content, were found increased on aging; an increased content of oxidation products is considered an effective aging factor, especially in the brain, with a majority of postmitotic cells. Mitochondrial enzyme activities, mitochondrial nitric oxide synthase (mtNOS), NADH dehydrogenase and cytochrome oxidase, behaved as markers of brain aging. The decrease in enzyme activities was directly related to the content of oxidation products and to the loss of neurological function in aged mice, this latter determined in the tighrope and the T-maze tests. The above mentioned conditions that increased mice lifespan were effective to decrease the level of oxidative stress markers, and to retard the decreases in mitochondrial enzyme activities and neurological function associated to aging. The activities of mtNOS, NADH dehydrogenase and cytochrome oxidase may be used as indicators of the effectiveness of anti-aging treatments.

P13-168

SYNTHETIC DIPEPTIDE (Cys-Gly): A NEW ANTIOXIDANT ACTIVE INGREDIENT THAT CAN BE OF GREAT USE IN ANTI-AGING SKIN CARE PRODUCTS

A. Plantivaux,[1] *E. Bauza,*[1] *T. Marchand,*[1] *E. Roux,*[1] *C. Dal Farra,*[1] *and N. Domloge*[1]

[1]***Vincience Research Center, Sophia Antipolis, France***

The skin is constantly exposed to environmental stress, such as UV irradiation, and is therefore the primary target for reactive oxygen species. As recent data has demonstrated a significant decline of antioxidant activity in photoaged skin, we were interested in investigating the protective effect of the synthetic dipeptide on cultured human cells exposed to UVB stress. Our studies showed that the application of the synthetic dipeptide at 1% enhanced SOD expression in UV-stressed cells, compared to the controls. This result corroborates the catalase assays that showed that dipeptide-treated cells exhibited a higher level of catalase activity in response to UV stress. Interestingly, the protective anti-oxidative stress effect of the dipeptide was supported by the significant decrease in protein carbonylation, and an enhanced cell viability, this protective effect was confirmed by comet assay that showed a remarkable decrease (73%) of DNA damage. These results demonstrate the great anti-oxidative effect of the synthetic dipeptide, and its considerable application in skin anti-aging products.